

# A FILTERABLE AGENT IN DUCKS

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Early in August 1952 an infection among ducks appeared on a farm near Headingly, Manitoba. The condition appeared serious enough to warrant the attention of Dr. R. H. Lay and other members of the Health of Animals Division who conducted an investigation. The symptoms were predominantly those relating to the central nervous system, such as twisting of the head, circling and walking backward and incoordination of movement. As reported, the infection appeared in ducks of about one month of age, while those of earlier hatches were not affected. Mortality was about 20 percent but morbidity was considerably higher. Approximately 5,000 chickens and 1500 geese which intermingled with the affected ducks remained healthy. In close proximity, but housed separately were 4,000 healthy turkeys.

Specimens were sent to Dr. Alfred Savage of the Provincial Veterinary Laboratory at Winnipeg, for autopsy and bacteriological study. According to Dr. Savage's report most cases revealed the presence of a thin layer of fibrinous exudate adherent to the external surface of the liver that was easily peeled off. The pericardium was often swollen and either adherent to the epicardium or filled with a clear gelatinous exudate. About 5 percent of the affected birds showed a unilateral sinusitis. The sinuses contained inspissated caseous purulent material odourless and greyish-yellow in color. Negative findings were reported on bacterial examination. Because the symptoms presented were not unlike those observed in Newcastle disease, although it was recognized that ducks and geese are considered to be resistant to this infection, spleen and brain tissues and sinus exudate were forwarded to the Animal Diseases Research Institute for virus study.

## EXPERIMENTAL STUDIES

Pools of tissues were finely minced in a tissue grinder and diluted 1:20 with sterile nutrient broth. The sinus exudate was diluted similarly. Before inoculation into embryonating chicken eggs, penicillin and streptomycin were added to control bacterial contamination. Eggs were inoculated on the chorio-allantoic membrane, in the allantoic sac, yolk sac, and intravenously, and examined daily. Embryo deaths occurred in all groups from 20 to 40 hours after inoculation. Following death, each egg was chilled, opened and closely examined.

The dead embryos revealed no apparent variation in size, but the entire external surface presented general congestion rather than specific areas of haemorrhage. The embryo livers appeared intensely swollen and friable. The chorio-allantoic membranes were oedematous without specific macroscopic lesions. An "agent" was found present which is described later.

Harvested allantoic and amniotic fluids were found to agglutinate normal chicken and duck red blood cells. Because the addition of positive Newcastle

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disease serum failed to inhibit this agglutination, the possibility of Newcastle disease was ruled out. Later, chicken, turkey, geese and duck sera obtained from the affected premises were examined serologically but without evidence of Newcastle disease infection.

#### ATTEMPTS TO IDENTIFY THE "AGENT"

The "agent" has been through twelve serial passages in chicken eggs and eight passages in duck eggs with no variation in serological results. All inoculi have been checked regularly and found bacteriologically sterile. Emulsions of egg tissues and fluids made up in 1:100 sterile physiological saline solutions were put through various grades of filters. The "agent" was demonstrated by egg inoculation, in filtrates from Berkefeld "coarse" and "medium" filters, but was not found in filtrates from a Berkefeld "fine" filter or a Seitz "EK" filter pad.

Titration studies in embryonating chicken eggs indicated that the "agent" in egg fluids up to  $10^{-6}$  and chorio-allantoic membrane emulsions up to  $10^{-7}$  was embryocidal.

Guinea pigs, rabbits, mice and rats inoculated by various routes with the original specimen material and the egg-propagated "agent" failed to demonstrate evidence of infection.

Chickens and ducks from one day old to mature age have been inoculated subcutaneously, intramuscularly, intravenously, intranasally and intratracheally without establishing infection. Intradermal inoculation by multiple skin stabbing likewise gave negative results.

The sinuses of chicks, ducklings and turkey poults were inoculated with the original specimen material and egg-propagated "agent" without producing infection or even local inflammatory reactions.

Sera obtained from chickens and ducks inoculated with the "agent" were later found to contain neutralizing antibodies. This was demonstrable in the haemagglutination-inhibition test when homologous antiserum inhibited the normal chicken red blood cell agglutination caused by the "agent". This serum failed to inhibit similar agglutination by the Newcastle disease virus. Neutralization could also be shown in embryonated eggs when inoculated with the serum-"agent" mixture. Antibody production was only transient, however, disappearing within four to six weeks after inoculation.

Smears of the chorio-allantoic and yolk sac membranes of infected chicken and duck eggs were stained by the Giemsa and Macchiavelli methods. Rickettsiae could not be demonstrated. The bluish counterstain of both stains however revealed small pleomorphic bodies in bacilli, coccoid and pear-shaped forms that were not found in similar tissue smears from normal embryonating eggs. This observation was suggestive of the bodies frequently observed in the pleuropneumonia-like group of organisms. Special medium was inoculated but no growth occurred.

#### SUMMARY

1. An "agent" possessing many properties of a virus has been isolated from brain, spleen and sinus exudate of ducks which presented symptoms suggesting involvement of the central nervous system.

2. This "agent" can be propagated in embryonating chicken and duck eggs and is capable of agglutinating normal chicken cells. Agglutination is not inhibited by Newcastle disease antiserum.

3. Specific antiserum was found to inhibit the haemagglutination of chicken red cells caused by this "agent". This serum failed to inhibit the agglutination caused by Newcastle disease virus.

4. Laboratory experimental mammals, chickens, ducks and turkeys failed to develop symptoms following inoculation of the original tissue and exudate suspensions or the egg-propagated "agent".

5. Preliminary studies failed to demonstrate that the "agent" is a rickettsia. Bluish stained pleomorphic bodies have been observed by staining with Giemsa and Macchiavelli methods. While these bodies are suggestive of the pleuro-pneumonia group, growth was not obtained on appropriate media.

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### DR. J. G. JERVIS

Dr. J. G. Jervis passed away in St. Paul's Hospital, Vancouver, B.C., on May 8th, 1953, aged 62 years.

Dr. Jervis was born in Vancouver, B.C., and graduated from the Ontario Veterinary College in 1912 and then entered general practice in Vancouver, B.C., in partnership with Dr. M. Sparrow. Later he practised for many years in Langley Prairie, B.C.

During his career he held official positions in the Health of Animals Branch as a part-time veterinarian, an accredited veterinarian and as a full time Veterinary Inspector, and at the time of his recent illness was on a rabies control assignment in Northern B.C.

He was President of the British Columbia Veterinary Association in 1924 and 1925, and served many years on the Council, and was a member of the C.V.M.A., A.V.M.A. and U.S. Sanitary Livestock Association.

He leaves a widow, and two daughters, Mrs. R. Winters of Langley Prairie, and Mrs. M. McCarrige of North Vancouver, B.C. and four grandchildren.